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The effect of saliva substitutes on enamel erosion in vitro

Aykut-Yetkiner, Arzu ; Wiegand, Annette ; Attin, Thomas

Abstract: OBJECTIVES To investigate the effect of saliva substitutes on enamel erosion in vitro. METHODS A total of 204 bovine enamel samples were embedded in acrylic resin and allocated to 17 groups (n=12). The specimens were eroded in an artificial mouth (3 days; 6×30 s/days, flow rate: 2 ml/min) using citric acid (pH: 2.5). Immediately after the erosive attacks, saliva substitutes (12 sprays, 3 gels) were applied. Between the erosive cycles the specimens were rinsed with artificial saliva (flowrate: 0.5 ml/min). A SnCl2/AmF/NaF-containing mouthrinse was used as positive control, water spray served as negative control. Enamel loss was measured profilometrically and the data were analyzed using one-way ANOVA followed by Scheffé's post hoc tests ($p<0.05$). RESULTS Four saliva substitutes increased enamel erosion, probably due to the low pH or the content of citric acid. Several saliva substitutes were able to reduce enamel erosion significantly by 60-90% (in the range of the positive control). The protective potential of these products was in the range of the positive control (reduction of enamel loss to 30% of negative control). The erosion-protective potential of these high-viscous products is probably related to their film-forming properties, leading to a mechanical protection of the surface. CONCLUSION Saliva substitutes containing a very low pH exhibit a distinct erosive potential, while most high-viscous products present an erosion-protective effect. It can be recommended that patients suffering from xerostomia and at high risk for dental erosion should use high-viscous saliva substitutes, but should avoid saliva substitutes with low pH or containing citric acid. CLINICAL SIGNIFICANCE It can be recommended that patients suffering from xerostomia and at high risk for dental erosion should use high-viscous saliva substitutes, but should avoid saliva substitutes with low pH or containing citric acid.

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The effect of saliva substitutes on enamel erosion: in vitro

Short title: Saliva substitutes' effect on erosion

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Keywords: enamel erosion, saliva substitutes, dry mouth

Abstract

Objective: To investigate the effect of saliva substitutes on enamel erosion *in vitro*. **Material and methods:** A total of 204 bovine enamel samples were embedded in acrylic resin and allocated to 17 groups (n=12). The specimens were eroded in an artificial mouth (3 d; 6 × 30 s/d, flow rate: 2 ml/min) using citric acid (pH: 2.5). Immediately after the erosive attacks, saliva substitutes (12 sprays, 3 gels) were applied. Between the erosive cycles the specimens were rinsed with artificial saliva (flowrate: 0.5 ml/min). A $SnCl_2$/AmF/NaF-containing mouthrinse was used as positive control, water spray served as negative control. Enamel loss was measured profilometrically and the data were analysed using one-way ANOVA followed by Scheffé's post-hoc tests ($p < 0.05$). **Results:** Four saliva substitutes increased enamel erosion, probably due to the low pH or the content of citric acid. Several saliva substitutes were able to reduce enamel erosion significantly by 60 to 90% (in the range of the positive control). The protective potential of these products was in the range of the positive control (reduction of enamel loss to 30% of negative control). The erosion-protective potential of these high-viscous products is probably related to their film-forming properties, leading to a mechanical protection of the surface. **Conclusion:** Saliva substitutes containing a very low pH exhibit a distinct erosive potential, while most high-viscous products present an erosion-protective effect. It can be recommended that patients suffering from xerostomia and at high risk for dental erosion should use high-viscous saliva substitutes, but should avoid saliva substitutes with low pH or containing citric acid. **Clinical significance:** It can be recommended that patients suffering from xerostomia and at high risk for dental erosion should use high-viscous saliva substitutes, but should avoid saliva substitutes with low pH or containing citric acid.

INTRODUCTION

Saliva plays an important role in oral health, and xerostomia is considered as an important risk factor for the development of oral diseases, such as caries or mucosal infection.[1]

Xerostomia is defined as the subjective complaint of oral dryness and is associated with quantitative and qualitative changes in saliva, while the feeling of dry mouth can also be present in patients with normal saliva production. Xerostomia can be caused by a variety of medications, autoimmune disease (Sjogren's syndrome), radiotherapy or chemotherapy for head and neck cancer, hormone disorders and infections. The feeling of dry mouth might impair the overall quality of life, due to difficulties in speaking, chewing and swallowing, altered taste and oral soreness and burning.[2] The prevalence of xerostomia is reported to be approximately 20%, with increased prevalence in older patients.[1,3]

Patients suffering from xerostomia have an increased risk of developing caries lesions.[4] As a low salivary flow rate and insufficient buffering capacity will prolong clearance time of acids and periods with a low intraoral pH, the risk of dental erosion might also be increased, especially in patients with a high intake of acidic drinks or a high vomiting frequency.[5,6,7]

Numerous saliva substitutes in forms of sprays, gels, oils, mouthrinses, pastilles, viscous liquids and gums are available to relieve the symptoms of dry mouth and increase salivary flow.[8] They each differ with respect to their base substance, chemical composition and viscosity. It is still questioned whether they are really effective for relieving the sensation of dry mouth and whether one topical therapy is superior to another.[9] Moreover, some salivary substitutes might increase dental erosion risk by decreasing intraoral pH significantly.[10,11,12]

So far, the effect of saliva substitutes on dental erosion was not investigated systematically. Therefore, the aim of this study was to investigate the effect of various saliva substitutes (spray and gels) on enamel erosion *in vitro* and to compare the effect

with $SnCl_2$/AmF/NaF mouthrinse with known anti-erosive potential. The tested null hypothesis was that saliva substitutes would not have a anti-erosive effect on enamel erosion.

MATERIALS & METHODS

Specimen preparation

A total of 204 cylindrical enamel specimens (diameter: 3 mm) were prepared from the labial surfaces of freshly extracted, non-damaged bovine incisors of 2-3 years old cattle, which were stored in 0.1% thymol solution for a maximum of 6 months at 5°C until used. The enamel specimens were prepared using a water-cooled trephine bur and embedded in acrylic resin blocks (Paladur, Heraeus Kulzer, Germany) each containing three enamel specimens. The unique shape of the resin blocks with a round tip on one end and a cornered tip on the other allowed exact repositioning of the specimens in the brushing machine as well as in the profilometer. The enamel surfaces were then ground and fine ground with water-cooled discs (1200, 2400 and 4000 grit, Water Proof Silicon carbide Paper, Struers, Erkrath, Germany). The specimens were cleaned with water and examined under a stereomicroscope (10X magnification) to ensure that they were free of surface cracks, decalcification or any sign of previous grinding. They were randomly allocated to 17 groups with n = 12 specimens each. The sample size of n = 12 was determined based on a pilot experiment. This power calculation was based on a difference in means of 1.9 µm detected at 88% power, considering that the standard deviation is 1.4 and using a two group t-test with a 0.05 two sided significance level.

Experimental Procedure

The de-/remineralization cycle was performed in a so-called artificial mouth[13] allowing alternating erosion and remineralization of the specimens under standardized conditions in chambers, which are connected to two multichannel pumps (acid solution and artificial saliva). The specimens were subjected to the artificial mouth for 3 days with six erosive

attacks daily. Each day, specimens were rinsed for 1 h with artificial saliva[13] (flow rate: 1 ml/min), eroded for 30 s citric acid (pH: 2.5, flow rate: 2 ml/min) and again rinsed with artificial saliva for 15 s (flow rate: 0.5 ml/min). Then, the chambers were opened and the saliva substitutes were applied. For standardization reasons, all test materials were applied with the same volume. Application oft the sprays was kept constant by pumping them from a fixed distance of 2 cm on the enamel surface using the same dispenser.

This cycling was repeated 6 times daily, and the specimens were kept in artificial saliva overnight. The artificial saliva used was prepared following the formulation (0.002 g ascorbic acid, 0.030 g glucose, 0.580 g NaCl, 0.170 g $CaCl_2$, 0.160 g NH_4Cl, 1.270 g KCl, 0.160 g NaSCN, 0.330 g KH_2PO_4, 0.200 g urea, 0.340 g Na_2HPO_4 in 1000 mL distilled water) given by Klimek et al.[14] and was renewed each day. The degree of saturation of the artificial saliva with respect to calcium-containing compounds (e.g. dicalcium phosphate dehydrate, DCPD; octa calcium phosphate, OCP; Hydroxyapatite, HA) was calculated with a microcomputer program[15] and amounted to DCPD: 1.24, OCP: 1.76 and HA: 7.59.

The contents, manufacturers, the viscosities and the pH levels of the 15 saliva substitutes are presented in Table 1. The pH-values of the saliva substitutes were checked using a pH meter (Metrohm, Herisau, Switzerland), and viscosities were measured with a viscometer (Becker Research Equipment, Göttingen, Germany) accordingly to Seeliger *et al.*.[16] The maximum detection limit of the standard solutions for determining viscosity amounted to 127 mm^2/s. Due to the high viscosity of several products, it was decided not to calculate the degree of saturation as they are usually only calculated for aqueous solutions and it can not be excluded that the ingredients of the saliva substitutes alter the functions of many variables.[17]

To standardize the volume of the saliva substitute sprays, all products were transferred to standard spraying bottles (BSC Industry Co., Ltd., China, 50 ml). The sprays were applied at a fixed distance of 2 cm from the specimens' surface (one pump: 0.14 g). The same volume was used when the gel products were applied. The excess layer was

immediately removed using a cotton swab; a thin layer was left on the surface. As soon as the test materials were applied, the chambers were closed and the next cycle was started.

A $SnCl_2$/AmF/NaF-containing mouth rinse (0.14 g, elmex Erosion Protection, Gaba, Switzerland) with known anti-erosive potential was used as positive control by dropping the solution on the specimens. Water spray was used as negative control.

Surface Profilometry Measurement

Enamel loss was measured using a contact profilometry (Perthometer S2, Mahr, Göttingen, Germany). The device is equipped with a custom-made jig for repositioning the appliances with the samples for successive measurements.

Substance loss was calculated based on the differences between baseline and final profiles with a custom-designed software (4D Client, University Zurich, Zurich, Switzerland). Five profiles were performed on each specimen with a distance of 100 µm between each profile via scanning from the reference surface to the eroded surface. The profiles were obtained by moving the diamond stylus across the dentin surface and the references areas. All resin blocks had a notch, which fits the metal jig of the profilometer table preventing the rotation of the specimen and allowing repositioning and the software allowed exact superimposition of the reference areas (acid-resistant acrylic resin).[18]

Statistical analysis

The normal distribution of the data was tested using Kolmogorov–Smirnov and Shapiro-Wilk tests. As data were normally distributed, one-way ANOVA followed by Scheffé's post-hoc tests were applied. The level of significance was set at $p < 0.05$.

RESULTS

Means and standard deviations of each group in terms of enamel loss are presented in Table 2. While test groups treated with Mouth Kote, Saliva natura, Stoppers 4 and Thayers increased enamel loss compared to the control, several sprays (Aldiamed, EMOFLUOR, Glandosane, Oasis, Saseem) and biotene oral*balance* gel reduced enamel loss significantly by 60 to 90%. Thereby, the protective potential of these products was in the range of the positive control (reduction of enamel loss to 30% of negative control).

DISCUSSION

Saliva substitutes with varying ingredients, pH levels and viscosities were tested in this study. It was shown that the application of four mouth sprays even increased the erosive loss, while six products significantly decreased erosive enamel loss to the range of the positive control. These products might be recommended for patients with dry mouth in terms of prevention of enamel erosion.

In the present study bovine enamel was used, as relative rather than absolute differences were of interest and only slight differences between human and bovine substrate exist.[19] The cycling model in the artificial mouth allowed for standardized rinsing with citric acid and artificial saliva at constant flow conditions. The flow rate in the artificial mouth during erosion with citric acid was applied in order to mimic oral conditions during consumption of a beverage.[20] Since it was suggested that exposure to the acidic environment without salivary interaction should not exceed a period of 2 min/cycle, the specimens were subjected to 6 x 30 s of erosion daily. These in vitro conditions simulate intra-oral real-life conditions as closely as possible.[21] Citric acid was chosen according to the composition of typically erosive beverages.[22]

Artificial saliva instead of human saliva was used, as large amounts of saliva with a consistent composition were necessary. Artificial saliva has been shown to act as an effective agent for rehardening of softened dental enamel in vitro.[23,24] Artificial saliva was shown to be oversaturated with respect to enamel, thus it can be anticipated that artificial

saliva would enhance remineralization of the softened enamel as mentioned by Tschoppe and Kielbassa.[25] Moreover, it considered as an appropriate medium in laboratory erosion experiments not least as human saliva might show a high intra- and intersample variability, and components of human saliva might rapidly be degraded or altered under in vitro conditions.[21]

Water served as negative control and was also pumped on the specimens' surface to exclude that any effect of the test sprays is related to the physical effect of spraying rather than to the chemical composition of the sprays. A $SnCl_2$/AmF/NaF-containing mouthrinse was used as positive control, as it was recently shown to have a distinct anti-erosive effect in clinical studies.[26]

The presence of the salivary pellicle was not considered in the present study. The salivary pellicle is known to reduce erosive demineralization.[27,28] However, to minimize the variables in this study and as relative rather than absolute differences were of interest, a salivary pellicle was not formed on the specimens' surface.

Enamel loss was significantly increased by saliva substitute sprays, which either exhibited a very low pH (pH < 4, Mouth Kote, Stoppers 4) or contained citric acid (Saliva natura, Thayers). However, previous studies found a remineralizing effect of Saliva natura on artificial carious lesions, but in these studies the calcium and phosphate content was increased compared to the native product.[29,30]

The erosion-protective potential of the sprays aldiamed, EMOFLUOR, Glandosane, Oasis and Saseem and of the biotene oral*balance* gel might be attributed to a physical barrier on the enamel surface due to film-forming properties of the products.[31]

Some previous papers have already shown that Glandosane has erosive potential.[32,33] However, these studies were focusing on the effect of saliva substitutes against demineralization rather than erosion. In contrast to the results of the present study, Glandosane was shown to have some demineralizing potential at least when specimens were stored several weeks in the respective solution. [32,33]

All saliva substitutes with a distinct erosion-protective potential exhibited a very high viscosity (>4 mm^2/s) due to various thickening agents (glycerol, cellulose, oil). Moreover, EMOFLUOR spray contains 750 ppm fluoride, which might increase the rehardening of erosively demineralized enamel[14] However, as the degree of saturation with respect to enamel was not calculated due to the high viscosity of most products, a correlation between the saturation of the products and the amount of enamel loss cannot be done.

Within the limitations of this study, citric acid-containing saliva substitutes or products containing a very low pH exhibit a distinct erosive potential, while most high-viscous products present an erosion-protective effect, probably due to their film-forming properties.

As numerous chemical factors, like pH, titratable acidity, degree of saturation, kind of acid and chelating properties have been identified to influence the erosive potential, the saliva substitutes being undersaturated with respect to HA and solutions with higher titratable acidity should have a demineralizing effect. However, further investigations are required to determine this phenomenon.

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calcium-modified acidic candies in irradiated dry mouth patients. *Oral Health Prev Dent.* 2010;**8**(2):173-178.

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Table 1. Composition, manufacturers, viscosity and pH of the different saliva substitutes (The maximum detection limit of the standard solutions for determining viscosity amounted to 127 mm2/s)

Tested materials Brand name	**Composition**
Mouth Sprays	
Aldiamed	Aqua, Propylene glycol, Xylitol, Glycerol, Microcristalline cellulose, Panthenol, Carboxymethyl cellulose, Sodium, Sodium benzoate, Lactoferrin, Disodium EDTA, Lysozyme, Hydrochloride, Aroma, Aloe Barbadensis
Biotene	Water, glycerin, xylitol, PEG-60 Hydrogenated castor oil, VP/NA copolymer, Aroma, Sodium Benzoate, Xanthan gum, Methylparaben, Propylparaben sodium saccharin, Cetylpyridinium Chloride, Limonene
EMOFLUOR	Aqua, Glycerin, Sorbitol, Maltitol, Ammonium phosphate, Hydroxyethyl cellulose, Ammonium fluoride, (750 ppm F) Methylparaben, Sodium saccharin, Sodium chloride, Potassium chloride, Propylparaben.
EvoDry	Evaux thermal spring water, Glycerin, Aroma, Cocamidopropyl, Betanamide MEA chloride, Sodium saccharin, Phenoxyethanol, Methyl paraben, Butyl paraben, Isobutyl paraben, Ethyl paraben, Propyl paraben, Piroctone olamine, Citric acid
Glandosane	Potassium chloride, Sodium chloride, Magnesium chloride, Magnesii chloridum, Calcium chloride, Potassium monohydrogen phosphate, Carboxymethylcellulose sodium, Sorbitol
Mouth Kote	Xylitol, Sorbitol, Yerba Santa, Citric Acid, Natural Lemon-lime Flavor, Vitamin C, Sodium benzoate, Saccharin, Sodium, Water
Oasis	Cetylpyridinium chloride, Copovidone, Flavor Methylparaben, PEG-60 Hydrogenated castor oil, Propylparaben Sodium benzoate, Sodium saccharin, Water, Xanthan gum, Xylitol
Rain Spry	Purified water, Xylitol, Aloe Vera concentrate, Vegetable glycerin, Natural Spearmint Flavouring, Calcium glycerophosphate cellulose Gum & Grapefruit Seed Extract as a preservative.
Saliva natura	Water, Sorbitol, Ascorbic, Citric acid anhydrous, Sodium hydroxide, Yerba Santa, Xylitol, Sodium benzoate, Lemon flavor
Saseem Mundspray	Water, Xylitol, Dexpanthenol, Carrageenan, Potassium sorbate, Sorbic acid, Sodium chloride, Potassium chloride, Potassium dihydrogen phosphate, Calcium chloride, Magnesium chloride, Sodium monofluorophosphate
Stoppers 4	Water (Aqua), Glycerin, Xylitol, Hydroxyethylcellulose, Lysozyme, Lactoferrin, glucose oxidase, Spearmint (Natural), Sodium benzoate
Thayers	Purified water, Vegetable glycerin, Calcium gluconate tris amino, Citric acid, Potassium chloride, Natural Peppermint Flavor

Gels	
Biotene oral*balance*	Lactoperoxidase, Lysozyme, Glucose oxidase, Lactoferrin, Inactive Ingredients: Hydrogenated starch hydrolysate, Xylitol, Hydroxyethylcellulose, Glyceryl polymethacrylate Beta-D-Glucose, Aloe Vera, Potassium thiocyanate
GC Dry Mouth Gel	Diglycerol, Aqua, Cellulose gum, Carrageenan, Sodium citrate Flavor, Ethylparaben, Limonene, linalool
Orajel	Active Ingredients: Glycerin (18%), Inactive Ingredients: Calcium disodium EDTA, Citric acid, Disodium phosphate, Flavor, Methylparaben, Water (Purified), Sorbitol, Sucralose, Thione Complex (A Patented Proprietary Blend), Xanthan gum
Control groups	
Erosion Protection	Aqua, Glycerin, Sodium gluconate, PEG-40 Hydrogenated castor oil, Olaflur-Aminfluorid (125 ppm F^-), Aroma Menthol, Stannous chloride, Sodium fluoride, Cocamidopropyl betaine, Sodium saccharin, Hydrochloric acid
Water	

Table 2. Enamel loss (µm, mean ± standard deviation) at the end of the experiment. Groups that are not significantly different from each other are marked with the same letters

Tested materials **Brand name**	**Enamel loss** **µm**
Mouth Sprays	
aldiamed	**1.53 ± 0.54[a]**
biotene	**1.75 ± 0.61[a,b]**
EMOFLUOR	**0.46 ± 0.21[a]**
EvoDry	**1.65 ± 0.47[a,b]**
Glandosane	**0.83 ± 0.46[a]**
Mouth Kote	**8.61 ± 2.98[f]**
Oasis	**0.36 ± 0.31[a]**
rain Spry	**1.98 ± 0.97[a,b]**
Saliva natura	**6.35 ± 1.03[e,f]**
Saseem Mundspray	**0.54 ± 0.40[a]**
Stoppers 4	**4.56 ± 1.00[d,e]**
Thayers Dry Mouth Spray	**4.24 ± 1.26[c,d,e]**
Gels	
biotene oral*balance*	**1.04 ± 0.68[a]**
GC Dry Mouth Gel	**1.68 ± 0.80[a,b]**
Orajel	**2.15 ± 0.61[a,b,c]**
Control groups	
Erosion Protection	**1.25 ± 0.51[a]**
Water	**3.82 ± 1.37[b,c,d]**